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Antibacterial Activities of Iron Chelators against Common Nosocomial Pathogens

Mitchell G. Thompson, Brendan W. Corey, Yuanzheng Si, David W. Craft,* and Daniel V. Zurawski

Walter Reed Army Institute of Research, Silver Spring, Maryland, USA

The activities of iron chelators (deferoxamine, deferiprone, Apo6619, and VK28) were evaluated against type strains of *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Escherichia coli*. Deferiprone, Apo6619, and VK28 each inhibited growth in standard and RPMI tissue culture medium, while deferoxamine had no effect. Additionally, time-kill assays revealed that VK28 had a bacteriostatic effect against *S. aureus*. Therefore, these newly developed iron chelators might provide a nontraditional approach for treatment of bacterial infections.

Iron is an essential cofactor of biochemical pathways in both prokaryotic and eukaryotic species. Numerous studies have assessed the potential viability of iron chelators as therapeutic agents against various microbes, but with only mixed success (4, 6–8, 10–17, 20, 24, 25, 27). Nevertheless, as novel iron chelators are developed for treatment applications such as neurodegenerative diseases (3, 9, 19) or β-thalassemia (3, 9), an evaluation of their antimicrobial activities should be tested, because their efficacies against bacteria may be superior to chelators previously tested. In the case of multidrug-resistant (MDR) bacteria, where entire classes of antibiotics are no longer treatment options (1, 21), iron chelators that have already undergone toxicity and preclinical testing in animals might provide an alternative treatment approach. MDR species, such as *Staphylococcus aureus* or *Acinetobacter baumannii*, are exceedingly difficult to treat because of nosocomial spread and infections in immunocompromised patients (1, 18, 21). The same microbes have also been responsible for wound infections incurred by military personnel who are immunocompromised after polytrauma (2, 23).

Defersirox and deferoxamine are approved by the U.S. Food and Drug Administration (FDA) but have demonstrated limited efficacies in combating bacterial infections (4, 6, 17). Deferoxamine is a siderophore, a molecule secreted by bacteria to capture iron; therefore, many bacteria challenged with deferoxamine also harbor a receptor capable of capturing such molecules when complexed to iron (4, 6, 17). Defersirox, while rationally designed to bind iron, failed to treat fungal infections (20) and is considered toxic (11).

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In this study, we sought to assess the antibacterial effects of iron chelators that have yet to be tested against bacteria, as well as deferiprone, a chelator recently FDA approved for iron overload due to blood transfusions in patients with thalassemia. Deferiprone has also been shown to have antibacterial properties against certain bacterial species *in vitro* (4, 10). Deferiprone (ApoL1) and Apo6619 were provided by ApoPharma, Inc., and VAR10100 (VK28 dihydrochloride) was provided by Varinel, Inc. Because of their iron chelation properties, both Apo6619 and VK28 (and their derivatives) are currently being studied for treatment applications (9, 19, 22, 28). Deferoxamine mesylate salt (DFO) and 2,2′-bipyridyl (DIP) were purchased from Sigma-Aldrich Inc. and were evaluated for comparison purposes.

TABLE 1 MICs of iron chelators against ATCC type strains grown in CAMHB

Bacterial species	Strain no.	MIC of iron chelator (μg/ml)				
		DIP	DFO	ApoL1	Apo6619	VK28
A. baumannii	17978	64	>512	128	256	128
A. baumannii	19606	64	>512	128	256	128
S. aureus	25923	512	>512	>512	>512	256
S. aureus	43300	256	>512	>512	>512	256
P. aeruginosa	PAO1	256	>512	256	>512	>512
P. aeruginosa	27853	256	>512	>512	>512	>512
K. pneumoniae	BAA-2146	256	>512	256	>512	>512
K. pneumoniae	700603	512	>512	512	>512	>512
E. coli	35718	64	>512	512	256	>512
E. coli	43888	64	>512	512	256	>512

Bacterial type strains considered common nosocomial infectious agents were acquired from the American Type Culture Collection (ATCC): *A. baumannii* (19606 and 17978), *Pseudomonas aeruginosa* (PAO1 and 27853), *S. aureus* (43300 and 25923), *Klebsiella pneumoniae* (BAA-2146 and 700603), and *Escherichia coli* (35718 and 43888). The MICs of DIP, DFO, ApoL1, Apo6619, and VK28 were determined by following the microdilution methodology recommended by the CLSI (5) in cation-adjusted Mueller-Hinton broth (CAMHB) against the bacteria listed above. The MICs were also determined in RPMI 1640 tissue culture medium (Life Technologies, Inc.), which may better represent the human host environment, with limited amounts of cofactors such as calcium, magnesium, zinc, and iron. Time-kill assays against *S. aureus* and *E. coli* were performed as described by White et al. (26). An initial inoculum of ~1.0×10^7 CFU/ml was challenged with either 1× or 2× the MIC of VK28, and cells were grown at 37°C for 24 h. Samples were

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Address correspondence to Daniel V. Zurawski, daniel.zurawski@amedd.army.mil.

* Present address: David W. Craft, Microbiology, Penn State Milton S. Hershey Medical Center, Hershey, Pennsylvania, USA.

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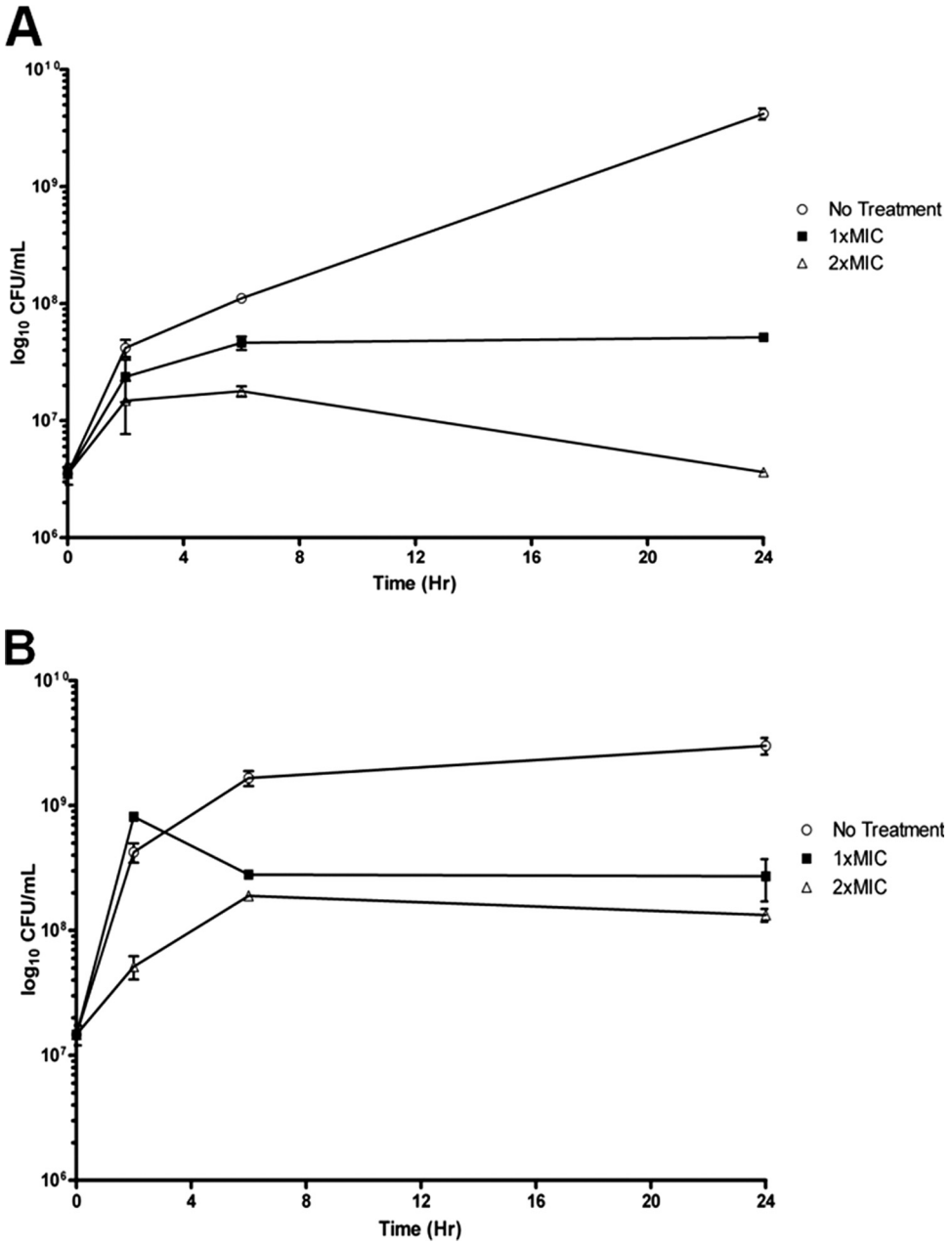

FIG 1 Time-kill studies of VK28 against *Staphylococcus aureus* 43300 (A) and *Escherichia coli* 48333 (B). The no-treatment results represent growth in the absence of chelator. The detection limit was 1.0×10^3 CFU/ml.

taken at 0, 2, 6, and 24 h, and the CFU/ml was determined via dilution and plating with a spiral plater (Advanced Instruments, Inc.), with each sample diluted over 3 logs onto plates containing CAMHB medium and agar. Biological replicates for all tests were performed at least three times in triplicate (technical replicates).

DFO did not affect bacterial growth in CAMHB (MIC, >512 μg/ml for all bacteria tested) (Table 1). The result was not surprising, since the compound may readily deliver iron to bacteria with a cognate siderophore receptor. In contrast, VK28 inhibited the growth of *A. baumannii*, *E. coli*, and *S. aureus* in CAMHB. Further, both ApoL1 and Apo6619 inhibited the growth of some strains of *P. aeruginosa* and *K. pneumoniae*, as well as *E. coli* and *A. baumannii*, while no effect on *S. aureus* was observed (Table 1). Because CAMHB is a rich broth with excess iron, carbon sources, and other cofactors far exceeding the levels in the human body, RPMI medium was chosen to evaluate the activity on the same bacterial strains in a more restrictive medium. When challenged with the iron chelators that showed the most promise in CAMHB, the MICs were reduced accordingly in this cofactor-limited environment (Table 2). When evaluated in RPMI, for VK28 the MIC improved 4- to 64-fold, and the ApoL1 and Apo6619 MICs improved 2- to 4-fold (Table 2).

Previous studies with iron chelators have demonstrated a bacteriostatic effect on bacterial growth (10, 14, 16). We performed a

TABLE 2 MICs of iron chelators against ATCC type strains grown in RPMI 1640 medium

Bacterial species	Strain no.	MIC of iron chelator (μg/ml)				
		DIP	DFO	ApoL1	Apo6619	VK28
A. baumannii	17978	32	>512	64	64	32
A. baumannii	19606	32	>512	128	128	8
S. aureus	25923	NA[a]	NA	NA	NA	32
S. aureus	43300	NA	NA	NA	NA	16
P. aeruginosa	PAO1	256	>512	128	512	16
P. aeruginosa	27853	256	>512	512	>512	16
K. pneumoniae	BAA-2146	128	>512	256	512	16
K. pneumoniae	700603	256	>512	256	512	16
E. coli	35718	64	>512	512	256	32
E. coli	43888	64	>512	256	256	8

[a] NA, not attempted.

time-kill assay to see if this was also true of the iron chelators evaluated in this study. *E. coli* and *S. aureus* were both challenged with either 1× or 2× the determined MIC for VK28 (Fig. 1). Growth of *S. aureus* was attenuated somewhat by both concentrations (Fig. 1A). Additionally, VK28 proved to have a bacteriostatic effect on *E. coli* at 2× the MIC (Fig. 1B). Similar bacteriostatic effects were observed for ApoL1 and Apo6619 (data not shown).

Unlike the mild effects observed with DFO and other iron chelators in previous studies (4, 9), VK28, ApoL1, and Apo6619 had pronounced effects on the growth of nosocomial bacteria. The outcomes observed could be related to the rational design of these iron chelators. For example, VK28 includes a piperazine ring that enhances polarity to cross the blood-brain barrier for the treatment of neurodegenerative disease (19, 28). It is possible that this polarity may also allow the chelator to cross certain bacterial membranes. ApoL1, in contrast, is a very small, neutral molecule, and these properties are known to facilitate its passage across host cell membranes and perhaps bacterial membranes as well. Therefore, in each case, the free iron might be chelated both inside and outside the bacteria, explaining the enhanced efficacy. Continued studies on these molecules, including combinatorial therapies with conventional antibiotics and animal modeling, will attempt to uncover the mechanisms by which these iron chelators provide a potent antimicrobial effect.

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